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Amorphous formulation and *in vitro* performance testing of instantly disintegrating buccal tablets for the emergency delivery of naloxone

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ABSTRACT The aim of this study was to develop a freeze-dried buccal tablet for the rapid delivery of naloxone in opioid overdose. The tablet composition was optimized to produce an amorphous matrix, which was confirmed by the absence of peaks associated with crystallinity observed by differential scanning calorimetry and powder X-ray diffraction. Tablets with high gelatin content lacked adequate porosity. Mannitol was added to the formulation to bridge and intercalate gelatin's tight polymer aggregates, however sodium bicarbonate was also required to prevent crystallization within the tablets. A linear reduction in mannitol's re-crystallization enthalpy was observed with increasing sodium bicarbonate concentration ($\Delta_{\text{re-cry}}H = -20.3[\text{NaHCO}_3] + 220.9$; $r^2 = 0.9$, $n = 18$). The minimum sodium bicarbonate concentration for full inhibition of mannitol crystallization was 10.9% w/w. Freeze dried tablets with lower amounts of sodium bicarbonate possessed a crystalline fraction that PXRD identified as mannitol hemihydrate from the unique peak at $9.7^\circ 2\theta$. Mannitol's greater affinity for both ions and residual water rather than its affinity for self-association was the mechanism for the inhibition of crystallization observed here. The optimized tablet (composition mannitol 24% w/w (4.26 mg), gelatin 65% w/w (11.7 mg), sodium bicarbonate 11% w/w (1.98 mg) and naloxone 800 μg) formed predominantly amorphous tablets that disintegrated in less than 10 s. Optimized tablets were chemically and physically stable over 9 months storage at 25°C . As speed of drug liberation is the critical performance attribute for a solid dosage form designed to deliver drug in an emergency, a novel imaging based *in vitro* disintegration assay for buccal tablets was developed. The assay was optimized with regard to conditions in the buccal cavity: i.e. temperature $33\text{--}37^\circ\text{C}$, volume of medium (0.1–0.7 mL) and use of mucin-containing biorelevant medium. The disintegration assay was sensitive to temperature, medium volume and medium composition; naloxone tablet disintegration was extremely rapid, with full disintegration ranging

from 5-20 seconds. In conclusion, rapidly disintegrating tablets have been developed which are suitable for proof-of-concept clinical trial in humans to determine the pharmacokinetics of naloxone delivered via the buccal route.

INTRODUCTION

Opioid overdose causes an estimated 69,000 deaths per annum and is a major contributor to the global burden of disease [1]. Naloxone is a highly effective opioid antagonist but must be delivered rapidly to the systemic circulation of overdose victims, who may be unconscious, so as to prevent fatal outcome from respiratory depression [2]. Naloxone is a highly water soluble drug and has a LogP of 2.09 and pKa above 7 (Table 1). Existing published pharmacokinetic–pharmacodynamics studies of naloxone do not enable simple determination of the appropriate dose required to achieve a target concentration or concentration–time profile to maximize its antidote efficacy [3]. Speed of onset is critical to reverse the life-threatening respiratory depression which characterizes the opioid overdose [3]. Naloxone injection is currently licensed for intravenous (IV) and intramuscular (IM) administration and is available in two concentrations, 0.4 mg/mL and 1 mg/mL injectable solution. Licensing of naloxone has recently changed in the UK to expand access to naloxone as a medicine which is available in emergency situations [4]. Making naloxone available as an emergency medicine is in accordance with the position statements of the American Academy of Clinical Toxicology, the American College of Medical Toxicology, the American Association of Poison Control Centers [5], the American Medical Association [6] and the World Health Organization [7]. However, the use of injections in emergency situations is limited by the training required to administer these, ideally using aseptic technique, and syringes are not readily portable [8, 9]. Orally ingested dosage forms are

unsuitable as they are not easily administered to an unconscious patient and naloxone undergoes extensive first pass metabolism and has low bioavailability (< 1%) after oral administration [10].

Prototype improvised kits for nasal administration have been introduced in ambulance services and clinical trials, but the use of nasal naloxone remains off-licence as supporting pharmacokinetic data is lacking. There are reasons for caution about reliance on nasal naloxone, and concerns about the clinical adaptation of untested unlicensed formulations have been expressed [11]. Other non-injectable options need to be considered.

Buccal delivery provides an attractive alternative route of administration for the emergency delivery of drugs [12-14]. The application of a tablet to the inner cheeks of the oral cavity is simple and easily accessible to a non-specialist bystanders or healthcare professionals in emergency situations [12]. Following naloxone liberation from the dosage form, the buccal mucosa, a 40-50 cell (500-600 μm) thick stratified epithelium, provides the principal absorption barrier [15]. The vasculature of the buccal mucosa drains into the retromandibular, lingual and facial veins, which in turn drain directly into the internal jugular vein and, via the superior vena cava, into the systemic circulation [12, 16, 17]. The absorption of naloxone from the human buccal cavity is unknown, but the bioavailability of naloxone from the buccal administration in rats has been reported to be 70%, compared to 0.3% via the oral route due to the extensive first pass metabolism [10], with maximum plasma levels obtained within 15 min [10]. Sublingual administration of 2-8 mg of naloxone solution has been reported to precipitate opiate withdrawal in humans within 30 min [18].

Dosage forms for buccal delivery include (i) tablets and lozenges, (ii) films, wafers and patches, (iii) liquids, creams, gels, ointments, (iv) sprays, lozenges, chewing gum and mucoadhesive film [19]. Of these options, tablets provide the simplest, most portable and easily applied formulation as an emergency medicine, but for efficacy will be required to disintegrate immediately on application. Orally disintegrating tablets have recently become widely accepted dosage forms, especially for pediatric and geriatric patients and there are more than 55 products having marketing authorization in the United States, European Union and Japan [20]. However, despite their rapid disintegration, dissolution times for drugs from these formulations is often in minutes rather than seconds [21]. Development of formulations with ‘instant’ disintegration rates (e.g. ≤ 10 s) is required for emergency medicines; one strategy to achieve this is to exploit the physicochemical advantages of amorphous materials, which enhance disintegration rate and bioavailability due to their increased molecular mobility compared to corresponding crystalline material [22, 23]. However, a major problem with producing and maintaining amorphous form in a product is physical and chemical instability, i.e. reversion to crystalline structure [24, 25]. Production of solid amorphous medicines is typically complex because the quality attributes of the final product are difficult to control, which has proved a limitation for many early phase amorphous formulations entering clinical trials [26].

The aim of this study was to develop a safe, easily administered and quick-to-act buccal tablet containing naloxone. Table 2 summarizes the ideal or target properties of a buccal tablet for the emergency delivery of naloxone. A novel instant disintegrating tablet formulation was designed by modifying the ratio of gelatin, sodium bicarbonate and mannitol to produce an amorphous, but stable tablet by freeze drying. Freeze drying is frequently used as a manufacturing technique for pharmaceuticals, its application to instant disintegrating tablets, also referred to as freeze

dried wafers, is an established technology, e.g. an example known as Zydis[®] was commercialized in 1993 [27]. However, due to the ending of the Zydis[®] related patent, there has been an increase in the research activity associated with this dosage form, and other forms of freeze dried wafers and tablets [16, 21, 28]. Such research activity has created the need for better and more relevant methods for evaluating the performance of freeze dried systems, especially orally and instant disintegrating tablets. The authors consider that this evaluation must include the characterization of solid-state behavior and the measurement of disintegration times under more bio-relevant conditions than the current pharmacopeia methods describe.

The principles of molecular and material science [29] were used to design a formulation and manufacturing process that confers structural and physicochemical properties for optimal stability and performance. Speed of drug liberation is the critical performance or quality attribute for a solid dosage form designed to deliver drug in an emergency. At present there is not a pharmacopoeia method for quantifying disintegration for instant disintegrating tablets [16, 21, 28]. Therefore a buccal disintegration test was developed which proved to be: (i) discriminatory for quality control purposes, and (ii) bio-relevant with potential for future development as an assay to predict *in-vivo* performance.

MATERIALS & METHODS

MANUFACTURE OF INSTANT DISINTEGRATING TABLETS OF NALOXONE HCl

Instant disintegrating tablets were produced in the Pharmacy Manufacturing Unit of Guy's Hospital of the Guy's and St Thomas' NHS Foundation Trust. The materials control, manufacture and testing of the tablets were performed under Good Manufacturing Practice

conditions at the MHRA licensed manufacturing facility at GSTT (Authorization number: 11387). All equipment and instruments were calibrated, tested and maintained in accordance with ICH guidelines [30]. Feed solution for tablet preparation was prepared by disintegrating 0.780 g of pre-weighed gelatin powder (Fagron Ltd, 110 g bloom strength), 0.132 g of sodium bicarbonate powder (Fagron Ltd) and 2.931 g of mannitol 10 (Fresenius Kabi) in 40 mL of water for injection (WFI) held at 70°C. Once all excipients were fully dissolved, a further 40 mL WFI (room temperature) was added and the solution was allowed to cool to room temperature. Naloxone hydrochloride dihydrate (pharm-grade; Fagron Ltd) 0.0586 g was dissolved in the feed solution, which was made up to 100 mL. The same method was used in preparing feed solutions with varying mannitol:gelatin ratios, where the weights of each of the excipients were adjusted accordingly.

Empty wells of an aluminium blister (Zhejiang Xinfei Machinery Ltd), made and designed specifically for this study, were filled with 1.500 g of naloxone HCl feed solution. Filled blister wells were cooled down to -20°C to allow feed solutions to freeze and then were maintained at -20°C, above its primary glass transition (T_g '), for a 2 h annealing step. After annealing, the blisters were cooled down to -80°C. Frozen tablets were removed from the wells and placed into pre-cooled freeze drying vials (1 oz Clear Glass Universal Type 1) packed inside a temperature controlled freeze drying chamber, -40°C, and the drying chamber was sealed. A Bench top (Lyotrap freeze dryer; LTE Scientific Ltd). was used to perform the freeze drying cycle. This freeze dryer model does not provide shelf heating option, therefore a 5 day freeze drying cycle was initiated to ensure all ice within the tablets was sublimed under ≤ 0.01 mbar and $\leq -40^\circ\text{C}$ [31]. At the end of the freeze drying cycle, the drying chamber was backfilled with

nitrogen, allowing it to reach atmospheric pressure with the cooling unit on. The drying chamber was opened and the freeze drying vials were immediately sealed with rubber stoppers and screw lids while inside the drying chamber. The finished products were removed from inside the drying chamber and inspected for breakage or shrinkage.

Quality control tests were performed on each of three batches of manufactured tablets to ensure that they matched specification limits given in table 4. The critical limits were set to comply with the requirements of the British Pharmacopeia and European Pharmacopeia for oral dosage forms (mass/content/physiochemical properties). While other limits; such as dimensions, are for guidance, and are not required by the BP. Thus they have been set broadly, see table 4, and will likely be refined upon scale up and technology transfer of the process. Whilst the target yield was 20 units per batch; at each test point/event 10% of the batch was tested. Where two tablets from each batch were tested for the uniformity of weight and dimensions (using a digital calliper) and disintegration time using an adapted USP disintegration method; the method involved placing two tablets into a 25 mL water bath at 37°C and observing their disintegration, making a total of 6 tablets used for each test. Full tablet disintegration was classified for this USP disintegration test as the absence of particulate matter, i.e. a clear solution, achieved within 3 minutes or 180 seconds.

NALOXONE HCl HPLC ASSAY

A published reverse phase HPLC assay for naloxone HCl was used to confirm chemical stability [32]. The method utilized a C-18 Gemini-NX 5 µm reverse phase column, mobile phase of 32%

v/v methanol HPLC grade and 68% 0.1 ammonium acetate (pH 5.8), isocratic flow rate of 1 mL/min, column temp of 37°C and an injection volume of 20 µL. Absorbance was measured at 229 nm.

DIFFERENTIAL SCANNING CALORIMETRY

Differential scanning calorimetry studies were performed over a temperature range of -40 to 200°C using a DSC Q20 (TA Instruments, New Castle, DE, USA) with a refrigerated cooling accessory (RCS). The DSC cell was purged with 50 cm³/min dry nitrogen and the RCS was purged with 150 cm³/min nitrogen. The DSC cell was calibrated following the instrument manufacturer's guidelines. Experimental conditions for freeze dried tablets followed an equilibration at 25°C for 5 min, ramp to 200°C (10°C/min), followed by a ramp to 25°C (10°C/min) and a ramp to 200°C (10°C/min). Samples were analyzed in aluminium pin-holed hermetic pans. All experiments were repeated three times. The sample size used was approximately 5 mg, with the mass for each experiment recorded accurately on a six-figure balance, (Micro balance: Sartorius UK Ltd).

Experimental conditions for sub ambient differential scanning calorimetry studies on feed solutions followed an equilibration at 25°C for 5 min, ramp to -40°C (10°C/min), followed by ramp to 25°C (10°C/min).

ENVIRONMENTAL SCANNING ELECTRON MICROSCOPY

Samples were adhered to a conventional SEM stub and imaged using a FEI Quanta 200F microscope. The operating conditions were: vacuum pressure 200 Pa, HV 20kV, a gaseous secondary detector and a typical magnification of ×1000.

POWDER X-RAY DIFFRACTION (PXRD)

PXRD analyses were performed on Rigaku MiniFlex 600 diffractometer (Rigaku, Tokyo, Japan). The samples were spread on a zero background holder and placed on a spinner stage. The instrument produces Cu K α radiation (1.5418 Å) operated at a voltage of 40 kV and a current of 15 mA over a scan range 3-40° 2 θ with a step size of 0.01° 2 θ at a speed of 5°/min.

DIGITAL IMAGE DISINTEGRATION ASSAY

A digital image or photographic disintegration assay was developed to measure tablet disintegration in small volumes of medium in temperature controlled blisters. Disintegration was quantified using a gel imager to follow tablet disappearance. The disintegration vessel was a thermal-jacketed aluminium blister sheet with black-painted wells of the same dimension as those used for manufacturing the tablets (Figure 1). Disintegration medium was phosphate buffered distilled water (pH 7.3 \pm 0.2) or a synthetic saliva adapted from the SS5 USP recipe for artificial saliva [33], which consisted of distilled water, salts (NaCl = 8 g/L, KH₂PO₄ = 0.19 g/L and Na₂HPO₄ = 2.38 g/L) and mucin 2.16 g/L (from porcine stomach).

Assay temperature was adjusted by placing the whole apparatus in a temperature controlled water bath at the target temperature. Disintegration medium, 0.7 mL, was pipetted into the blister wells adjacent to the test well and micro probe thermocouples connected to a data logger thermometer were used to monitor the temperature of the disintegration medium. Once the

temperature of the disintegration medium in blister wells reached the target temperature the apparatus was placed inside a heat-insulating box of polystyrene and transferred into a closed box gel imager for the disintegration assay. This apparatus allowed accurate temperature logging throughout the disintegration assay to ensure the temperature of the disintegration medium was maintained $\pm 1^{\circ}\text{C}$ of the target temperature.

Disintegration was measured using a GeneSnap version 6.07.03 gel imager, with the camera located above test blister well. A reference image was then taken of test blister well containing disintegration medium, after which the well was dried and an instant disintegrating tablet was placed in the blister. An image of test well was then taken ($t = 0$ s) and the assay initiated by adding the required volume of temperature-conditioned disintegration medium onto the tablet, (e.g., 0.7 mL at 35°C) after which 100 consecutive images were taken at 0.4 s intervals. Image J analysis software, was used to analyze the images by determining the mean grey value (MGV), corrected for baseline at each time point and normalized to the assay range.

RESULTS

TABLET FORMULATION

Ratios of mannitol, gelatin and sodium bicarbonate were varied with the aim of identifying a tablet composition that would form an amorphous and porous freeze-dried product to meet the ideal properties described in table 2. Mannitol was utilized because of its hydrophilic nature, bulking properties and common use as a lyoprotectant [27]. Gelatin was selected to confer the ideal quality attributes for a successful product that meets the target properties of an ideal instant disintegrating tablet (Please refer to table 2 for the ideal properties); i.e. gelatin typically forms glassy amorphous complexes with relatively high glass transition temperatures, T_g 50-90°C, provides structural strength and has mucoadhesive properties [12, 27].

In the process of defining the collapse temperature of freeze-dried feed solutions, the behavior of a series of test formulation solutions were characterized during freezing by DSC; where the effects of varying the ratio of mannitol:gelatin on the T_g' of the aqueous solutions were investigated in the presence of sodium bicarbonate, a disintegrating agent, which reduced the T_g' of pure gelatin aqueous solutions by 14°C (Table 3). Results showed that high percentages of gelatin, in the test aqueous solutions, contributed to raising the T_g' , as test solutions with the lowest mannitol:gelatin ratio showed the highest T_g' (Table 3). Based on the T_g' values presented in table 3, the primary drying temperature was maintained well below the lowest T_g' , of -25°C shown by all of the test formulations. Thus the temperature within the freeze drying chamber was always below T_g' and the collapse temperature, approximately 2°C > T_g' [34].

Preliminary results showed that simply freeze drying aqueous solutions of pure mannitol and pure gelatin produced tablets of very poor quality. Freeze dried tablets composed of 100% w/w mannitol possessed a distinct melting peak with an average onset at $163.9 \pm 0.5^{\circ}\text{C}$ ($n=3$), indicative of a crystal melt (Figure 2). A small endotherm at approximately 60°C for 100% w/w freeze-dried mannitol was representative of the hemihydrate crystalline form of mannitol [35], indicating that freeze drying a solution of pure mannitol produced a crystalline product.

Comparable studies have demonstrated that freeze dried mannitol contains a mixture of the hemihydrate together with the anhydrous polymorphs as mannitol hemihydrate is quite unstable [35, 36]. The melting points for the α and β polymorphs are very close together and have been reported to fall between 165 and 166°C [37, 38]. A lower onset for this melting peak of approximately 164°C indicates the presence of an additional phase. Further inspection of the leading edge of the endothermic peak revealed a very small and broad inflexion overlaid on the rapidly falling heat flow curve. Nunes et al. have attributed this broadening of the peak onset to conversion of an anhydrous δ polymorph to the β form of mannitol. Dehydration of the hemihydrate form mannitol at 60°C initiates the formation of the δ polymorph [35]. The δ polymorph is enantiotropic and thus will undergo transition to the more stable β form over quite a wide temperature range, typically from 140 to 180°C with a relatively small peak area when observed by DSC, thus it is often difficult to identify this transition [38].

Pure mannitol crystalline tablets were brittle and difficult to remove from their sample vials without collapsing into a powder. In contrast, pure gelatin tablets had a sticky texture and lacked porosity. Formulations containing both mannitol and gelatin proved successful when freeze-dried; addition of gelatin diminished the crystalline melting peak in the freeze-dried product

whereas mannitol conferred porosity within the tablets. However, even with high gelatin content, persistent peaks were observed in the amorphous halo of the powder x-ray diffraction results. This indicated a crystalline fraction within the freeze-dried tablet and the unique peak at $9.7^\circ 2\theta$ identified the presence of mannitol hemihydrate [35], an example of this unambiguous peak can be seen in Figure 3a.

Since mannitol has an affinity for inorganic salts and this miscibility often leads to the inhibition of mannitol crystallization when present in freeze dried solids [38], sodium bicarbonate was introduced as a ternary agent. Physical mixtures of mannitol and sodium bicarbonate were investigated by heating to 200°C to melt the mannitol, then analyzed by DSC while cooling at $10^\circ\text{C}/\text{min}$ back to room temperature. The addition of sodium bicarbonate reduced the size of the mannitol re-crystallization peak in the cooling cycle. At concentrations of sodium bicarbonate above 10% w/w, crystallization of mannitol could no longer be seen. The reduction in the observed mannitol re-crystallization enthalpy was linear with increasing sodium bicarbonate concentration ($\Delta_{\text{re-cry}}H = -20.3[\text{NaHCO}_3] + 220.9$; $r^2 = 0.9$, $n = 18$). The extrapolated line crossed the enthalpy axis at a concentration of sodium bicarbonate at approximately 10.9% w/w (supporting information; Figure S1), indicating that this is the concentration of sodium bicarbonate at which crystallization of mannitol is inhibited entirely. Therefore, unless otherwise stated all freeze dried tablets were formulated to contain 11% w/w sodium bicarbonate. Scanning electron microscopy images revealed that the inclusion of sodium bicarbonate resulted in an increase in pore size but a reduction in the thickness of the pore walls compared to tablets prepared without sodium bicarbonate (data not shown).

The effect of mannitol:gelatin ratio on the crystallinity of the tablet was screened using differential scanning calorimetry. As the proportion of mannitol reduced relative to gelatin, progressively broader peaks with a reduced area appeared at lower temperatures. Peaks were entirely absent from formulations containing < 24% w/w mannitol (Figure 2). These observations were confirmed by X-ray diffraction (Figure 3a) indicating that the freeze-dried product containing 24% w/w mannitol and 11% w/w sodium bicarbonate was fully amorphous. Characterisation of the tablet excipients by PXRD showed distinct peaks for mannitol, naloxone and sodium bicarbonate, but no peaks for gelatin (Figure 3b). Thus, an optimized formulation, with a composition of 24% w/w mannitol, 65% w/w gelatin and 11% w/w sodium bicarbonate was defined and this has shown to meet ideal disintegration rate and optimal stability as described in table 2. These tablets were also confirmed to be predominately amorphous by PXRD, both with and without the incorporation of naloxone 800 µg (Figure 3b).

TABLET SPECIFICATION AND STABILITY

The instant disintegrating buccal tablets conformed reproducibly to quality specifications for weight, size, speed of disintegration and drug content (Table 4). The white hemispherical porous tablets were 29.4 ± 0.2 mm in length, 16.1 ± 0.5 mm in width with a depth of 3.0 ± 0.2 mm and weighed 17.7 ± 0.4 mg (Figure 4). Scanning electron microscopy (Figure 4) revealed the pore size in the tablet to have an average length of 0.23 ± 0.02 mm(SE) and an average width of 0.094

± 0.01 mm (SE), $n=20$. The target drug content, 800 μg of Naloxone HCl/tablet and chemical stability over 9 months when stored under nitrogen at 4°C or 25°C were confirmed by HPLC assay. The residual moisture content of the tablets was $10.4 \pm 3.2\%$ w/w, which is relatively high but it did not result in chemical degradation of the API or physical instability (Table 4).

IN VITRO DISINTEGRATION ASSAY FOR BUCCAL TABLETS

Imaging freeze-dried tablets using the adapted gel imager was relatively straightforward. Tablets of the optimized naloxone formulation appeared white but once the disintegration media had been added, a clear solution very rapidly developed revealing the black painted surface of the blister well beneath. For example, adding 0.7 mL of phosphate buffer to a naloxone tablet, with the temperature of the blister well held at 37°C, resulted in approximately half of the matrix remaining at 2s and by 10s the matrix had disappeared, figure 1. For the digital imaging disintegration assay the limit or target for tablet disintegration was classified as the time taken to achieve 10% of the matrix remaining or 90% disintegration. This target was met for the optimized naloxone tablets, under the conditions of 37°C and 0.7 mL, at 4.8 ± 0.6 seconds and furthermore by 10 seconds only 6% of the matrix remained, figure 5a & 5b. It should be noted that a very small amount of white colour remained in the images recorded at the end of disintegration assay. This was attributed to small air bubbles remaining in the solution and internal reflection at the curved edge of the blister well resulting from the position of the light sources. No particulate matter was seen when the contents of the blister well, from a typical naloxone tablet experiment, were viewed under a light microscope. Even under high magnification only air bubbles in a clear solution were observed. When the phosphate buffer was

replaced with artificial saliva, the very small amount of residual white colour was further diminished at the end of the naloxone tablets' disintegration. The surface-active nature of the mucin present in the saliva dispersed the air bubbles, and thus the percentage of matrix remaining fell to a reading of approximately zero, figure 5c.

The novel digital imaging disintegration assay was used to explore the effects of temperature, solvent volume and composition on the disintegration of the tablets. Under all conditions the tablets disintegrated fully (>90%) within 30 s. Tablets disintegrated in < 10 s in 0.7 mL of phosphate buffer at 35°C (Figure 5a). Temperature variation over the range reported to exist in the buccal cavity [39], 33-37°C did not alter the disintegration rate, but the rate was 4-5 times slower at 25°C. In opiate overdose, the volume of oral fluid available in the buccal cavity may be reduced compared to 0.7 mL in a typical adult human [28]. Reducing the amount of fluid available to the tablet progressively reduced the rate at which the tablet disintegrated, with disintegration in 0.1 mL being 4.5 times slower than in 0.7 mL (Figure 4b). Interestingly, when phosphate buffer was replaced with synthetic saliva, a slightly quicker disintegration rate was observed, a result of better spreading and wetting of the tablet caused by the mucin present in the disintegration medium (Figure 5b).

Evaluating the discrimination between disintegration profiles, measured at different temperatures and volumes, was performed using the similarity factor (f_2) test [40]. This approach was applied because of the high number of data points recorded for each disintegration profile; therefore making other common statistical approaches, for example the MANOVA analysis, impractical. This analysis confirmed that the digital imaging disintegration assay disintegration profiles were sensitive to the volume of disintegration medium used, for example the f_2 value comparing between a disintegration volume of 0.7 and 0.4 mL at 35°C was 28.36, (for this statistical

approach an f_2 value below 50 indicates low similarity or in other words a significant difference between the two profiles of data). Interestingly the profiles at different temperatures, indicated a high similarity for disintegration between 33, 35 and 37°C, as all comparisons had f_2 values equal or greater than 50, with only the profiles recorded at 25°C showing a statistical difference to the rest of the data set.

To validate the digital imaging disintegration assay, the marketed freeze-dried and orally disintegrating tablet, Imodium Instant® was investigated using 0.7 mL of phosphate buffer at 37°C. Imodium Instant® tablets showed slower disintegration compared to the naloxone tablets and failed to meet the target with 46.0 ± 0.2 % of the matrix remaining after 30 seconds, supporting information, Figure S2. Light microscopy aided the validation because it showed that the much higher percentage of the matrix remaining, detected in the disintegration assay, was caused by the presence of aggregated particles.

DISCUSSION

An instant disintegrating tablet aiming to rapidly deliver naloxone HCl was developed for a planned clinical trial associated with the emergency buccal delivery of an opioid antagonist in opioid overdose. The product is suitable for proof of concept clinical trials in humans to determine the pharmacokinetics of naloxone delivered via this route. The size and shape of the developed tablet were designed to ensure a high surface area contact with the buccal epithelium. The open pore structure and amorphous nature of the low density tablet, $11.8 \pm 0.3 \times 10^{-3}$ g/mL, were designed for rapid release of drug in low volumes of fluid. Additionally, the size and shape of the tablet were fashioned based on the recommendations of healthcare professionals

experienced in treating overdose patients [27]. These dimensions contrast with smaller orally disintegrating tablets currently marketed, for example Imodium Instants® have a diameter of 7.5 mm [27]. The developed naloxone instant disintegrating tablet is larger, at a length of 29 mm and a width of 16 mm to make it easier to insert into the buccal cavity of an unconscious patient using a fingertip.

A tablet specification was developed for weight, dimensions, rate of disintegration and drug content and used to verify batch-to-batch reproducibility and stability (table 4). Batch-to-batch verification was performed by testing 10% from each of 3 batches. However, this was repeated 10 times over the course of a 9 month stability study, thus all units within the 3 batches were tested to verify batch-to-batch. Tablets containing 24% w/w mannitol showed no shrinkage or collapse from their dimensions after the initial freezing step, confirming that the T_g ' of the formulation was not exceeded during the drying cycle. If the T_g ' of mannitol in maximally freeze concentrated aqueous solutions is exceeded during drying, collapse and crystallization of the amorphous cake is observed as a dramatic shrinkage of the product [36]. Mannitol in freeze drying solutions was shown to crystallize at temperatures close to -25°C (Table 3) [31]. The addition of small amounts of salt stabilized amorphous mannitol produced from freeze concentrated solutions by raising the primary glass transition by 2 degrees (Table 3) [31]; hence sodium bicarbonate was included in the tablet matrix of this study [31]. Furthermore, the inclusion of sodium bicarbonate enhanced the porosity of freeze dried material.

One of the roles of gelatin in the formulation is to raise the glass transition of both the freeze concentrated solution and the dried amorphous product in which gelatin maintains the

amorphous structure. The drying cycle reported within this paper is conservative, incorporating a wide safety margin; when considering scale up and increasing process efficiency, the drying temperature of -40°C could be increased.

Gelatin alone was insufficient to prevent crystallization of mannitol within the tablet during freeze drying. Even at 65% w/w gelatin, sodium bicarbonate was required to eliminate the persistent PXRD peak, indicative of the crystalline hemihydrate form of mannitol. This peak disappeared when 5% sodium bicarbonate was added to the feed solution. However, measurement of re-crystallization during the cool cycle using DSC revealed that concentration of sodium bicarbonate for full suppression of crystallization was 11% w/w. Thermal analysis, especially DSC, is influenced less by particle size and density issues compared to PXRD because the whole of the crystalline fraction present in the sample contributes to the response observed [41]. Thus DSC was more sensitive for determining the concentration of sodium bicarbonate needed to prevent mannitol crystallization. When salts are present in freeze concentrated solutions of mannitol, the sugar has a greater affinity for ions and water, than with itself and thus the formation of mannitol crystals is inhibited. However water is key to this mechanism; removal of a large proportion of the non-frozen water during secondary drying and this protective effect is lost and mannitol crystals are observed [38]. Thus secondary drying was negated in the preparation of the tablets reported here and a longer primary drying cycle was applied. This resulted in 10% w/w residual water content for the tablets that maintained their amorphous structure but did not adversely influence the stability of the product.

A wide range of mannitol to gelatin concentration ratios were investigated to allow selection of a predominantly amorphous formulation for further investigation. Gelatin acts as a binder and provides structural strength, resistance to breakage and a highly porous spongy structure [27]. Too much gelatin can have a negative effect on the rate of disintegration and dissolution as a result of inter-molecular interactions between the polymer chains of gelatin, through both hydrogen bonding and steric hindrance [12]. Both mannitol and salts are incorporated within freeze-dried materials to bridge and intercalate these tight polymer aggregates and thus expand the porous structure [42]. Thus, high concentrations of gelatin in the formulation increase the likelihood of strong inter-polymer chain attractions, resulting in water trapping and the subsequent formation of gels [27]. Unlike other studies, for example Gugulothu et al. [27], where this problem was overcome by lowering the gelatin content and compensating with crystalline mannitol as a supporting agent, we have shown that it is possible to increase the ratio of gelatin to mannitol and form amorphous tablets (Figures 2 & 3).

To circumvent the issue of strong gelatin intermolecular interaction, small amounts of an ionic salt, i.e. sodium bicarbonate, were used to expand the gelatin network, inhibit mannitol crystallization and generate an amorphous material [42]. This resulted in a fast disintegrating, gelatin-mannitol amorphous complex. Gelatin may also play an important role in the long term stability of amorphous mannitol by increasing the glass transition of amorphous mannitol by approximately 13°C, as reported by Kim et al. [41] Mannitol in turn acts as a lyoprotectant agent for proteins and is thus believed to stabilize the porous structure of gelatin in freeze-dried formulations [38]. The stabilizing effect of mannitol in freeze drying decreases with an increase in mannitol crystallinity [43], which is avoided in the current product.

Mannitol polymorphs were clearly detected in the DSC thermograms for the tablets composed of non-optimal ratios of mannitol and gelatin. For the crystalline containing samples, sodium bicarbonate also plays a significant role in the formation and thermal stability of the polymorphs observed. Telang et al. [38] have shown that salts will depress the melting points of mannitol polymorphs by up to 15°C, with additional broadening of the associated peaks. The complex thermal and PXRD results show that a mixture of the hemihydrate and β polymorphs are formed during freeze drying and the tablets are heated, de-hydration and conversion results in the observation of melting profiles for the δ & α polymorphs as well as the underlying β form. These transitions are broadened and depressed by the presence of sodium bicarbonate.

There is no standard method for *in vitro* disintegration tests for instant disintegrating tablets. Many studies use standard USP apparatus 2 disintegration methods with a disintegration volume of 900 mL, whereas the total volume of saliva in the mouth does not usually exceed 3 mL [44]. Other studies have attempted to study disintegration by applying physical force to the tablet using a texture analyzer [45], in which case disintegration profiles are therefore dependent on mechanical strength of the tablet rather than its disintegration properties. We report a novel and reproducible digital image disintegration assay to study rapidly disintegrating buccal tablets. The assay allows automated data collection to monitor tablet disintegration in a volume and temperature controlled environment, using conditions relevant to the buccal mucosa. The disintegration of the tablet as a whole is used as a surrogate for drug release, and was rapid, economical, discriminatory and appropriate for the product developed. A factor that may limit the wider application of the assay is its reliance on the disappearance of white colour as a measure of tablet disintegration, which would exclude its use for evaluating the disintegration of tablets with insoluble excipients. Combining image analysis algorithms, common to particle size

analysis, with our photographic approach to small volume disintegration is likely to remove this temporary limitation.

Adapting the GeneSnap gel imager and the Image J analysis software for the quantification of tablet disintegration was able to provide the necessary level of detail within each image to quantify disintegration, as the following evidence demonstrates. 1) The disintegration assay successfully discriminated between ODT's that fully disintegrated, e.g. the optimized naloxone formulation, and ODT's that partially disintegrated, e.g. the Imodium Instant® tablets, in the 0.7 mL of medium used to model the buccal cavity, figure 2s. 2) When the solution/dispersion generated by the disintegration assay was analyzed by light microscopy after 30 seconds, the optimized naloxone formulation formed a clear, particulate free solution, whereas the Imodium Instant tablet formed a dispersion of rectangular shaped crystals with an average length of 12 μm with an associated SE of 0.8 μm . Thus the novel image analysis approach could resolve partial disintegration when a dispersion of particles was formed as opposed to a clear solution, figure 2s. 3) Each image that contributed to the individual time point within the disintegration profile was relatively large at 2.76 MB. Such a high resolution permitted a wide range of observed mean grey values that were used to determine the % of the matrix remaining and thus the disintegration times. For example, the mean MGTV readings for a typical disintegration assay for the optimized naloxone formulation was 1770 ± 61 at the start, then 961 ± 56 at 3 seconds, 200 ± 5 at 10 seconds and 191 ± 4 at 30 seconds, (these were mean values determined using three separate tablets tested under identical conditions, \pm SE). MGTV readings at 10 and 30 seconds were very close to the background reading for a blister well containing only disintegration medium. There was typically an order of magnitude reduction in the MGTV readings from t zero to near complete

tablet disintegration, which is encouraging in terms of sensitivity towards quantifying tablet disintegration.

The novel disintegration assay was optimized for temperature in the range of 33-37°C, corresponding to buccal temperature range [39], and for disintegration medium with a volume in the range of 0.1-0.7 mL corresponding to saliva volumes range (Figure 5) [46]. No significant differences were observed in the disintegration profile over the buccal temperature range, but profiles were significantly different when the test was conducted at 25°C (Figure 4a).

Directly correlating the reduction in the mean grey value of a tablet's image to the disintegration of its freeze-dried matrix is not without complexity. For wider applicability, it should be noted that the current version of the gel imager records only in black and white, thus disintegration is recorded as a disappearance of the colour white. For coloured tablets an appropriate colour filter may be used on the camera to enhance the contrast in the grey scale before conversion into a black and white image. Another complicating factor is the inclusion of polymers that in their solid form are white but form translucent masses of high viscosity when exposed to water, e.g. cellulose based polymers, such as microcrystalline cellulose and hydroxyl propyl methyl cellulose. The authors would recommend in such cases that the image based disintegration assay should be augmented with inverted microscopy and the use of appropriate stains. For example, an aqueous based stain with an affinity for cellulose, e.g. the diazo dye, Congo Red, should be added to the blister well immediately after the disintegration assay. Congo red has a strong affinity for cellulose, therefore if aggregated cellulose polymers persist, the suggested stain would contrast boundaries and concentration gradients within the blister well. Thus if gel like structures are present, the use of inverted microscopy will allow detection of agglomerates that are small enough to permit an acceptable disintegration limit, or those extended aggregated

structures that indicate an absence of disintegration. The use of inverted microscopy allows these measurements to be conducted in-situ. Such elaboration on the digital imaging disintegration assay was unnecessary for the application reported in this paper because cellulose based polymers were not included in the naloxone tablet.

CONCLUSION

A novel amorphous instant disintegrating tablet, containing naloxone, was successfully produced to good manufacturing practice standards. The composition, based on three excipients, mannitol, gelatin and sodium bicarbonate, was essential for fulfilling the design aims for a naloxone instant disintegrating buccal tablet. The tablets were both chemically and physically stable for 9 months and may be used as a prototype in clinical trials for the instant buccal delivery of emergency naloxone. A novel disintegration method for the instant disintegrating buccal tablets was developed and used to investigate the disintegration profile of the tablet formulations manufactured in this study. The test has potential for development as a quality control test for instantly disintegrating buccal tablets and, with further exploration of biorelevant test conditions, an assay that is predictive of *in vivo* performance. Total disintegration of the naloxone buccal tablet was achieved in less than 10 s which is critical for their intended application.

Table 1. Details of the physical and chemical characteristics of Naloxone. Naloxone structure was constructed using ACD/ChemSketch.

Molecular Formula	C ₁₉ H ₂₁ NO ₄ [47]
Structure of Naloxone	
Melting Range	200-205°C [48]
Solubility	Freely soluble in water, soluble in ethanol 96%, practical insoluble in toluene [48]
Log₁₀ P (Octanol water partition coefficient)	2.09 [49]
pKa	7.9 [49]
Description	White or almost white, crystalline powder, hygroscopic [50]

Table 2 target properties of an ideal instant disintegrating tablet

Property and description	Target properties	Limits
Solid state	Predominantly amorphous matrix	Absence of peaks assisted with crystallinity in both DSC and PXRD
90% Disintegration time	< 10 seconds	Based on BP limits for instant disintegrating tablets < 3min
Drug content	0.8 mg	Based on BP limits for naloxone injections 0.76-0.84 mg
Size; to cover typical fingerprint area.	Length: 29 mm Width: 16mm	Length: 26-30mm Width: 14-18mm
Physical stability; size, and disintegration time	12 months , not less than a 5% change of the following: Size: Length: 26-30mm Width: 14-18mm 90%Disintegration time < 10 s	6 months , not less than a 5% change of the following: Size: Length: 26-30mm Width: 14-18mm 90%Disintegration time < 10 s
Chemical stability; Drug content	12 months , not less than a 5% change of the following: Based on BP limits for naloxone injection 0.76-0.84 mg	6 months , not less than a 5% change of the following: Based on BP limits for naloxone injection 0.76-0.84 mg

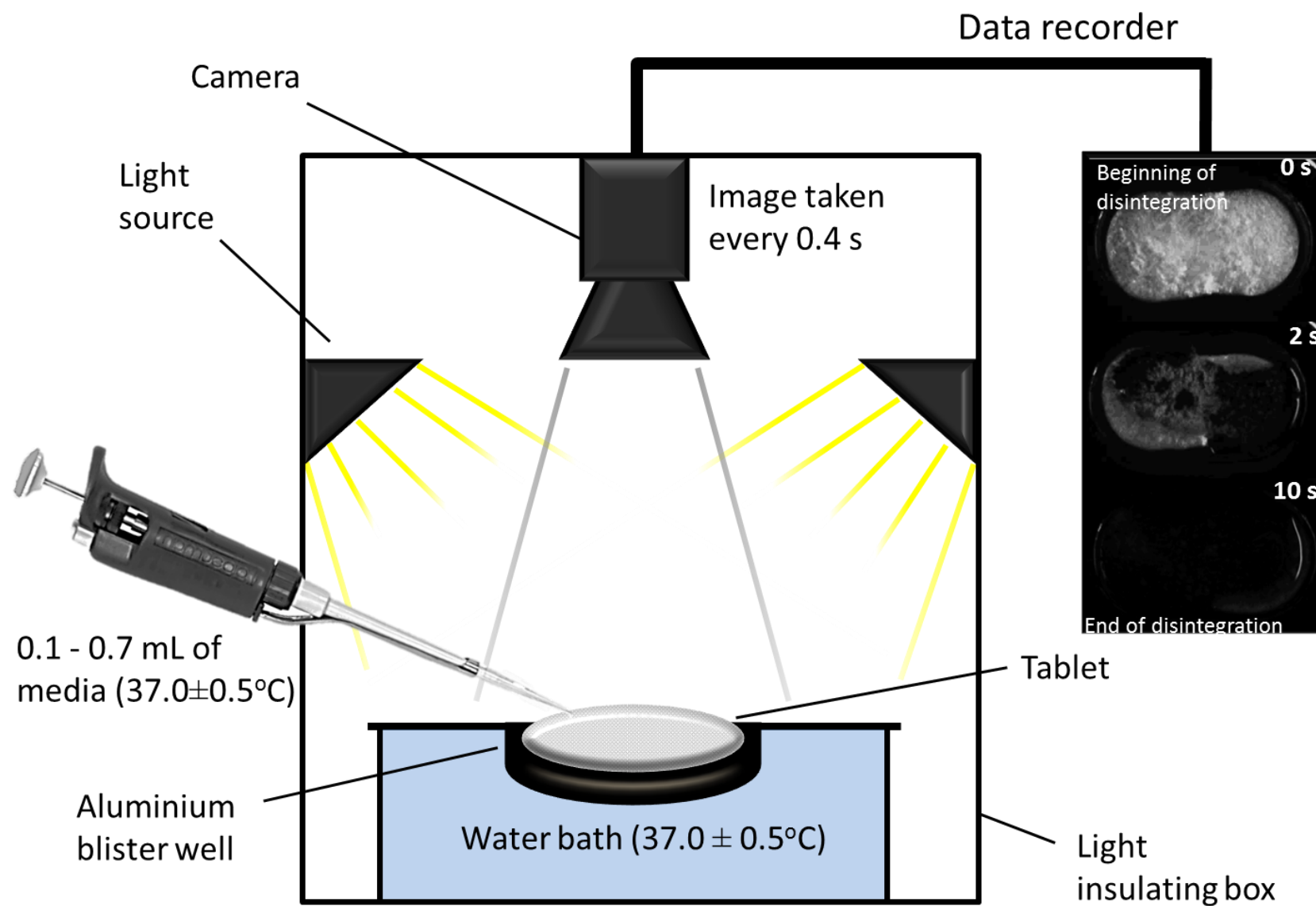


Figure 1. Schematic for the digital image disintegration assay, constructed from an aluminium blister sheet with a painted black background to provide contrast for the tablet. Disintegration of the tablet was monitored as the mean grey value using an image analyser. Controlled temperature maybe altered, in the present study temperatures in the range between 25°C and 37°C were investigated.

Table 3 Primary glass transition temperatures (T_g) of feed solutions containing varying concentrations of mannitol and gelatin in the absence and presence of sodium bicarbonate. The standard error is of n=3

Equivalent to w/w% composition per tablet			Average T_g /°C	SE
Mannitol	Gelatin	Sodium Bicarb		
0.0%	100.0%	0%	-9.1	0.03
0.0%	89.0%	11%	-23.1	0.18
24.0%	65.0%	11%	-22.8	0.13
44.5%	44.5%	11%	-25.2	0.40
65.0%	24.0%	11%	-24.3	0.22
89.0%	0.0%	11%	-23.0	0.13
100.0%	0.0%	0%	-25.0	0.10

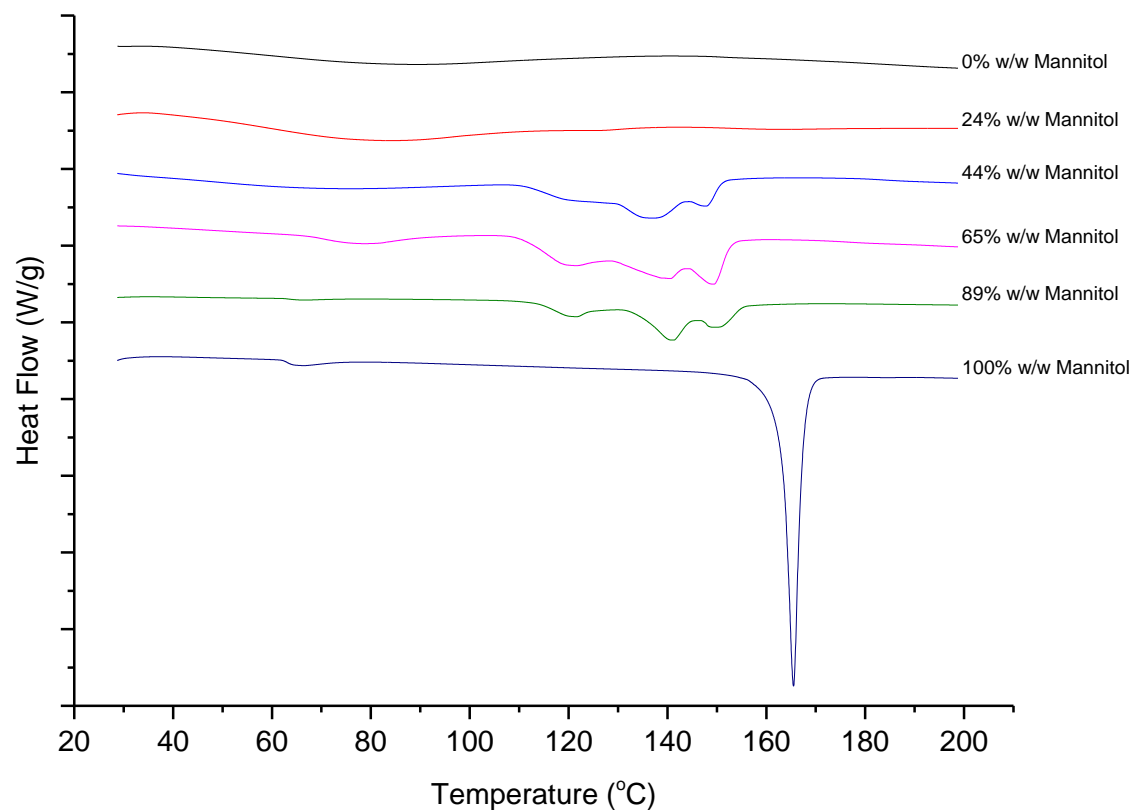


Figure 2. Differential scanning calorimetry to show the effect of mannitol:gelatin ratio on the thermal properties of the freeze dried instant disintegrating tablets. The tablets were composed of mannitol:gelatin in the ratios illustrated, plus sodium bicarbonate 11% w/w, with the exception of the 100% w/w mannitol sample.

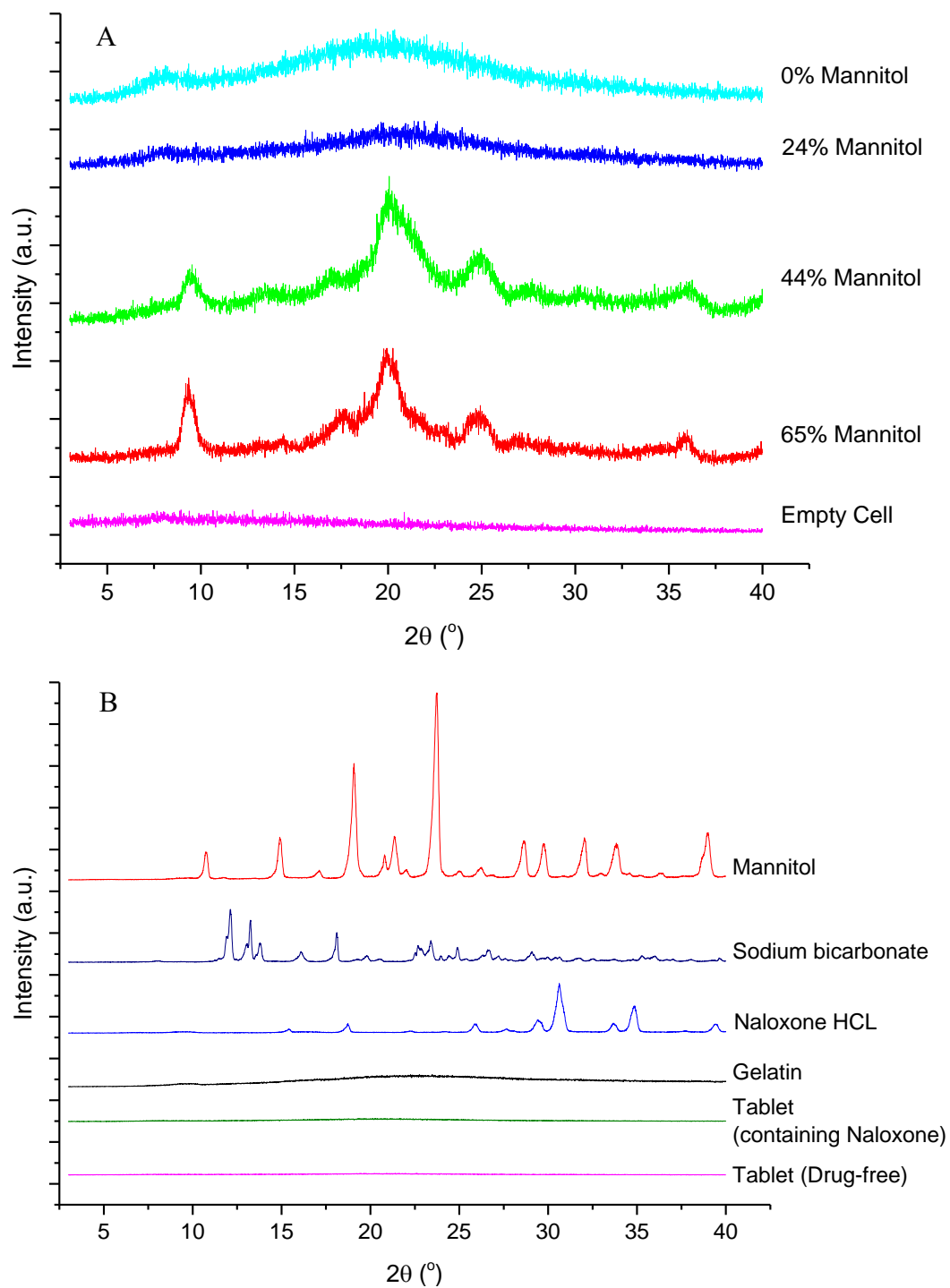


Figure 3. (a) Powder X-ray diffraction to show the effect of mannitol:gelatin ratio on the solid state properties of the freeze dried instant disintegrating tablets. The tablets were composed of mannitol:gelatin in the ratios specified, plus sodium bicarbonate 11% w/w. (b) Powder X-ray diffraction of individual tablet excipients, plus the formulated product with and without naloxone 800 μ g.

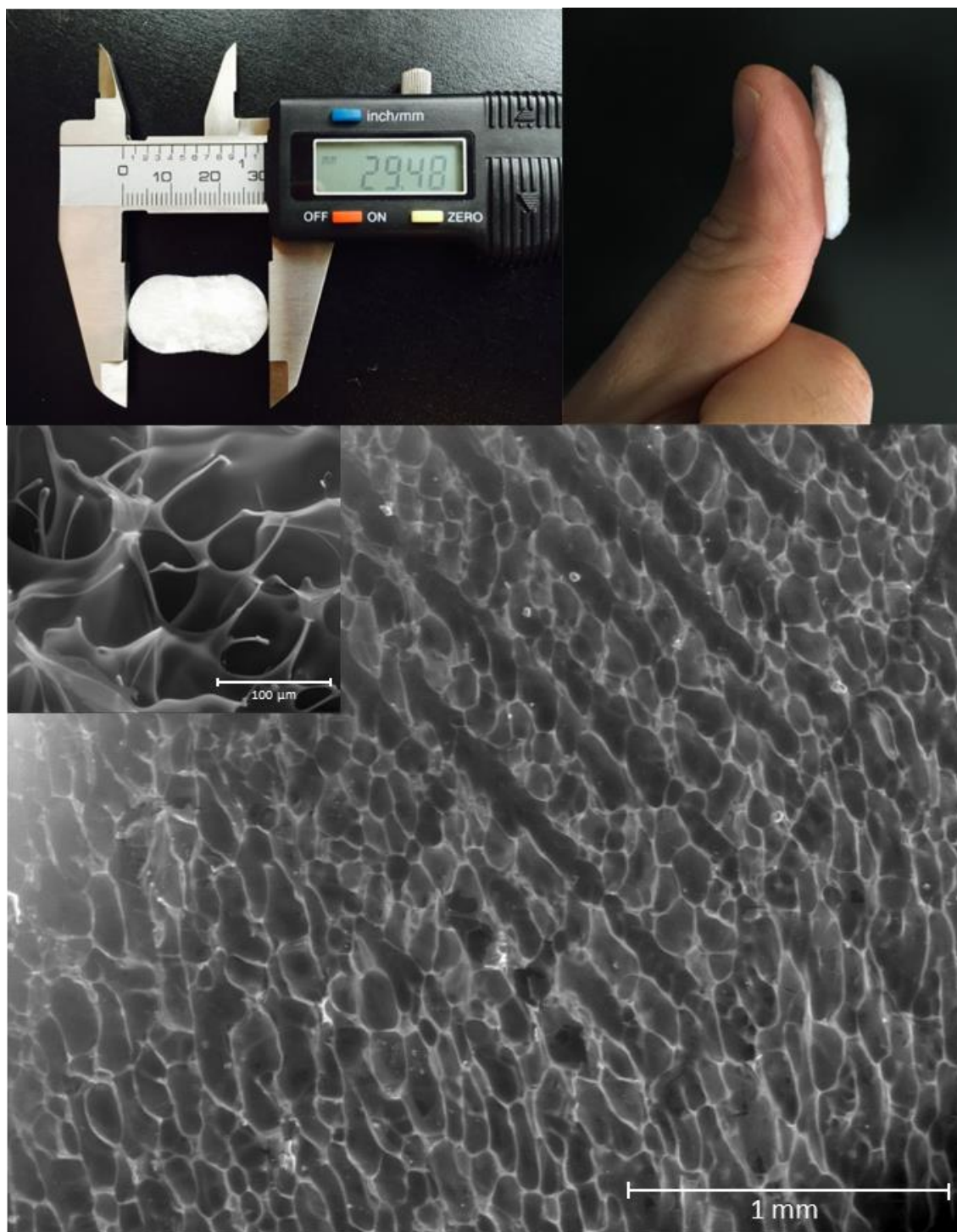


Figure 4. Top left, photograph of the naloxone instant disintegrating tablet with the length displayed in mm, top right showing the tablet's intended method of dispensing to an unconscious patient's buccal cavity. Bottom main image; presenting a scanning electron micrograph of the instant disintegrating tablet, with a smaller image to its top left corner; showing porous structure at higher magnification. Average pore length = 0.23 ± 0.017 mm (SE) and width 0.094 ± 0.006 mm (SE) (n=20).

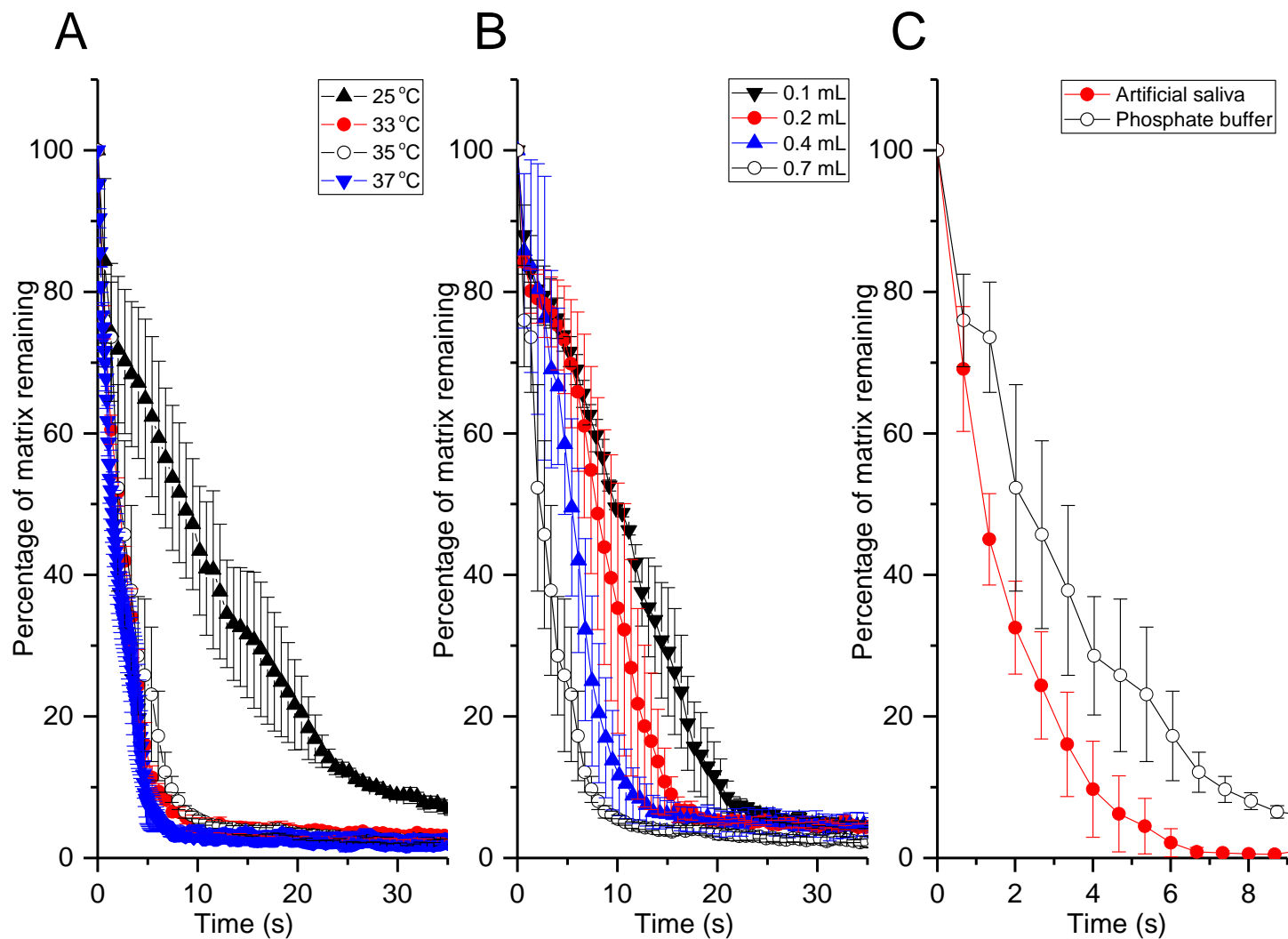


Figure 5. Effect of (A) temperature [volume 0.7 mL; medium – phosphate buffered saline], (B) fluid volume [temperature 35°C; medium – phosphate buffered saline], (C) disintegration medium [temperature 35°C; volume 0.7 mL] on the disintegration profile of the naloxone instant disintegrating tablet ; using a digital image disintegration assay. Data represent mean \pm standard error, n=3.

Parameter	Specification	Stability		
		0 months	9 months	9 months
			4°C	25°C
Tablet weight (mg)	16.9 - 20.7	17.8 ± 0.5	17.8 ± 0.5	17.6 ± 0.5
Dimension - length (mm)	26.0 - 30.0	29.4 ± 0.2	29.1 ± 0.3	29.1 ± 0.7
Dimension - width (mm)	14.0 - 18.0	16.1 ± 0.5	16.1 ± 0.3	16.0 ± 0.3
Disintegration test* (s)	≤180	14.0 ± 5.9	9.0 ± 5.0	10.0 ± 5.0
Naloxone HCl assay (mg)	0.76 - 0.84	0.80 ± 0.01	0.81 ± 0.02	0.80 ± 0.03

Table 4. Naloxone HCl instant disintegrating tablet specification and stability data after 9 months; 3 batches each n=2, thus 6 data points were used for each mean value with ± determined from the standard deviations. *BP adapted disintegration test

ASSOCIATED CONTENT

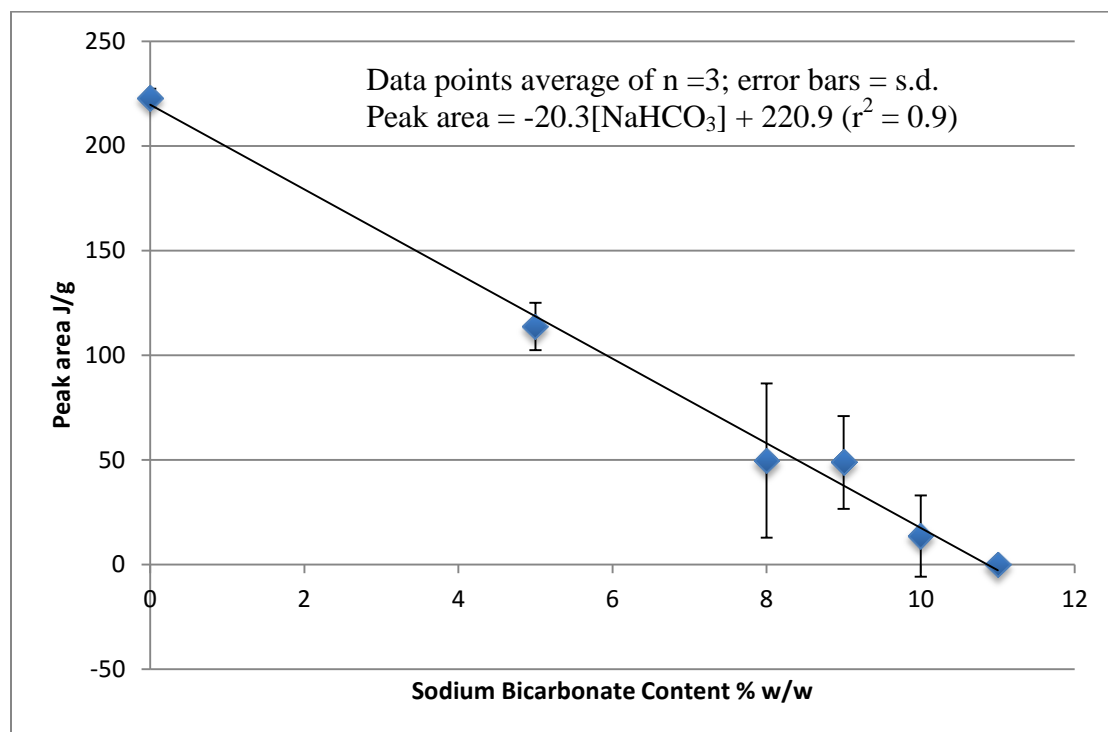


Figure 1s: Peak area of the mannitol recrystallization peak measured in the cooling cycle by differential scanning calorimetry against the concentration of sodium bicarbonate present in the binary physical mixtures.

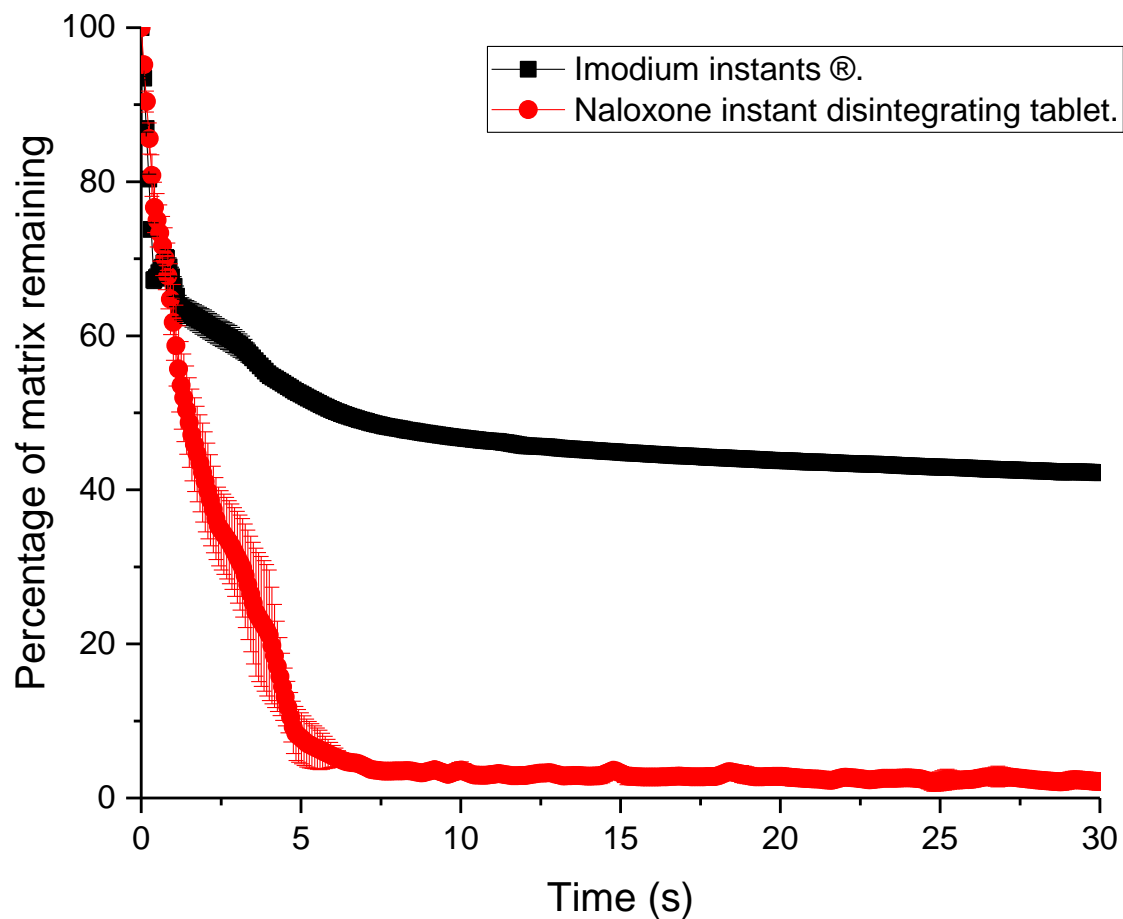


Figure 2s: Disintegration of the naloxone instant disintegrating tablet compared to a Zydis® based formulation, marketed as Imodium Instants® (containing 2mg loperamide HCl), at 37°C and 0.7 mL of phosphate buffered saline. The Imodium Instants® tablets produced a cloudy suspension with 46% of the matrix remaining at 30 seconds, while the novel naloxone instant disintegrating tablet generated an almost clear suspension with 10% of the matrix remaining 4.8 seconds and by 10 seconds only 6% of the naloxone instant disintegrating tablet matrix remained. Data represent mean \pm SE, n=3

Table 1S Summary of the f_2 test for disintegration profiles with different temperatures and volumes. $f_2 \geq 50$ shows high similarity between the profiles. Low similarities are only seen between 25 °C and other temperatures. While discrimination against volume shows low similarities between all volumes with the exception of 0.2 and 0.1 mL

Discrimination against temperature				Discrimination against volume			
Reference profile	Tested profile	f_2	Similarity	Reference profile	Tested profile	f_2	Similarity
37 °C	35 °C	57.6	High	0.7 mL	0.4 mL	28.36	Low
	33 °C	63.32	High		0.2 mL	19.13	Low
	25 °C	19.78	Low		0.1 mL	17.09	Low
35 °C	37 °C	57.6	High	0.4 mL	0.7 mL	28.36	Low
	33 °C	72.83	High		0.2 mL	35.26	Low
	25 °C	22.74	Low		0.1 mL	27.89	Low
33 °C	37 °C	63.32	High	0.2 mL	0.7 mL	19.13	Low
	35 °C	72.83	High		0.4 mL	35.26	Low
	25 °C	21.75	Low		0.1 mL	48.2	High
25 °C	37 °C	19.78	Low	0.1 mL	0.7 mL	17.09	Low
	35 °C	22.74	Low		0.4 mL	27.89	Low
	33 °C	21.75	Low		0.2 mL	48.2	High

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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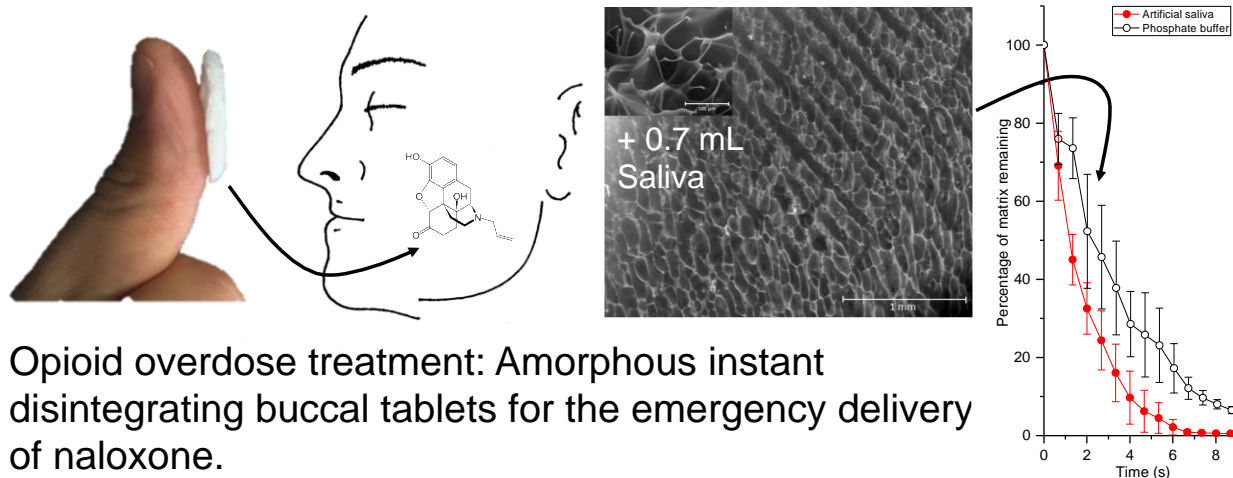
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Table of Contents Graphic and Synopsis



Opioid overdose treatment: Amorphous instant disintegrating buccal tablets for the emergency delivery of naloxone.